A hitchhiker's guide to survival finally makes CENs

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Most strains of the yeast *Saccharomyces cerevisiae* contain many copies of a 2-μm plasmid, a selfish autonomously replicating DNA that relies on two different mechanisms to ensure its survival. One of these mechanisms involves the high fidelity segregation of the plasmids to daughter cells during cell division, a property that is starkly reminiscent of centromeres. A new study reported in this issue (see Hajra et al. on p. 779) demonstrates that this high fidelity is achieved by the 2-μm plasmid, effectively recruiting the centromeric histone Cse4 from its host yeast cell to forge its own centromere and finally revealing how the 2-μm plasmid has survived in budding yeasts over millions of years.

Two strategies to avoid extinction

Since their original discovery, the 2-μm plasmids of yeast have provided a fascinating paradigm of selfish DNA. Typically present at a copy number of 60 per cell, they impose a perceptible cost in fitness to the host yeast cell. Yeast cells that have been cured of 2-μm plasmids (or Cir⁰ strains) have a growth rate advantage of 1.5–3% over their plasmid-containing counterparts (Cir⁺ strains; Mead et al., 1986). In spite of this fitness disadvantage to their hosts, 2-μm plasmids are ubiquitous in *S. cerevisiae* strains, and related plasmids are found in other highly divergent *Ascomycetes* species, implying that 2-μm–like plasmids have successfully hitchhiked in budding yeast cells for more than tens of millions of years.

They accomplish this remarkable evolutionary stability by using two different strategies to ensure their own transmission: partitioning and amplification (Velmurugan et al., 2003; Jayaram et al., 2004). After DNA replication, partitioning ensures a high fidelity of segregation of plasmid molecules. This requires two encoded proteins, Rep1 and Rep2, which bind specifically to a cis-acting *STB* (for stability) locus and, together, mediate this plasmid segregation. In the absence of such an active segregation mechanism (i.e., if plasmid segregation was simply diffusion dependent), the rate of loss of 2-μm plasmids from daughter cells coupled with the fitness advantage of Cir⁰ cells would lead to the rapid clonal extinction of 2-μm plasmids. In the case of a

decrease in copy number below a steady state value, plasmid amplification reestablishes this steady state by increasing rounds of replication. This amplification uses an elaborate series of recombination events mediated by the encoded Flp recombinase and two cis-acting *FRT* (*Flp recombinase target*) sites using a single DNA replication initiation event (Futcher, 1986; Volkert and Broach, 1986).

This redundancy of functions is perplexing for a selfish plasmid that is only 6 kb in length. One can easily imagine why amplification is necessary to spread to new host cells; in essence, this is a common property of all selfish elements, including transposons. Less obvious is why a selfish plasmid would go through the trouble of encoding an active partitioning mechanism if it had a robust amplification mechanism, given that the probability of a reduction of copy number from 60 to 0 in one cell cycle is extremely low. A partial answer emerged from cytological visualization of 2-μm plasmids in yeast cells, which revealed that these 60 individual plasmid molecules are present in just one to two clusters and that these clusters are inherited en masse (Scott-Drew and Murray, 1998; Velmurugan et al., 2000). Missegregation of these clusters can quickly lead to clonal extinction of the 2-μm plasmids. Indeed, the deletion of Rep1 or Rep2 can lead to a 30-fold higher loss of 2-μm plasmids relative to wild type after just seven to eight cell divisions (Bianchi et al., 1991). Thus, it is mostly by virtue of its partitioning function that the 2-μm plasmid avoids extinction.

A forged centromere

How does the 2-μm plasmid achieve this high fidelity of segregation? Clues began to emerge from previous studies that showed a striking genetic and cytological concordance of plasmid segregation to that of yeast chromosomes. First, 2-μm plasmids were found concentrated near the poles of the yeast mitotic spindle (Velmurugan et al., 2000), which is a cytological localization akin to yeast centromeres. Second, in an *ipl-2* yeast strain (*ipl* is the yeast Aurora kinase gene), both 2-μm plasmids and yeast chromosomes were found to missegregate in tandem fashion (Velmurugan et al., 2000). Finally, like yeast chromosomes, the 2-μm plasmid was found to recruit the yeast cohesin complex using Rep1 and Rep2, presumably to pair newly replicated plasmids (Mehta et al., 2002). This recruitment of cohesin was dependent on a specialized chromatin structure at the *STB* locus and on the chromatin remodeling activity of the RSC2 complex (Wong et al., 2002; Yang et al., 2004).

All of this circumstantial evidence had pointed to the 2-μm plasmid usurping the chromosome segregation machinery

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Figure 1. **Two modes of recruiting Cse4.** (A) Yeast chromosomal centromeres (*CEN*s) are 125 bp and contain highly homologous CDE I and III sequences and a CDE II (86–98% adenosine-thymidine rich) that are the same length but are variable in sequence across all centromeres. The CBF3 complex proteins bind to the CDE III element and help recruit Cse4 to assemble a single Cse4 nucleosome most likely at CDE II. For simplicity, only the CBF3 complex and Mif2 are shown. (B) The *STB* loci of 2-μm plasmids consist of proximal *STB* repeats (each repeat is ~60% adenosine-thymidine rich and 125 bp in length) and a distal *STB* element that acts as a transcriptional silencer and is important for *STB* function. No CBF3 complex proteins are known to localize to *STB*, and Cse4 recruitment at *STB* is independent of Ndc10 function. Instead, this recruitment relies on the plasmid-encoded Rep1 and Rep2 proteins and the RSC2 chromatin remodeling complex.
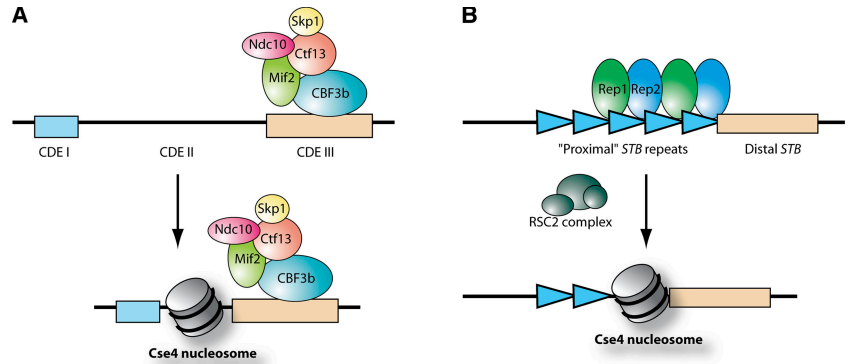
from the host yeast cell to ensure its own partitioning. Now, Hajra et al. (2006) add a critical piece to this puzzle by demonstrating that the 2-μm plasmid utilizes the yeast centromeric histone Cse4 (Stoler et al., 1995) to ensure correct segregation. Centromeric histones like Cse4 are exquisitely specific markers of centromeric chromatin in virtually all eukaryotes. The authors first show that Cse4 and 2-μm plasmids colocalize in chromosome spreads. Using chromatin immunoprecipitation followed by PCR, they show that Cse4 localizes specifically to the *STB* locus. Using high-salt extractions, they show that the *STB* locus of the 2-μm plasmid indeed assembles a Cse4-containing nucleosome, which protects the *STB* locus from restriction enzyme–mediated cleavage. They further demonstrate that *STB*-localized Cse4 is protected from proteolytic degradation in the same manner as *CEN* (yeast centromere)-localized Cse4 (Collins et al., 2004). Finally, they demonstrate that Cse4 is genetically required for the correct partitioning of the 2-μm plasmid. In the absence of wild-type Cse4, Rep2 does not localize to *STB* (although Rep1 does), RSC2 complex–mediated chromatin remodeling does not take place, and cohesin assembly at *STB* is blocked. Indeed, Hajra et al. (2006) argue that correct partitioning requires correctly remodeled *STB* chromatin containing a Cse4 nucleosome, which can nucleate Rep1–STB–

Rep2 interactions. These results together imply that the *STB* locus effectively mimics *CEN* function.

However, there are important differences between 2-μm plasmid and yeast chromosome segregation. The 3′ *CDEIII* element of *CEN* sequences specifically recruits proteins of the CBF3 complex, including Ndc10 (Goh and Kilmartin, 1993; Jiang et al., 1993), which in turn helps recruits a Cse4 nucleosome, most likely at the *CDEII* element based on genetic data (Fig. 1 A; Keith and Fitzgerald-Hayes, 2000). Thus, Cse4's localization to *CEN* sequences is disrupted in an *ndc10-1* mutant at nonpermissive temperatures, but its localization to *STB* loci is unaffected. Conversely, although Cse4 localization to *STB* loci requires Rep1 and Rep2 proteins (Fig. 1 B), the deletion of Rep1 or Rep2 has no effect on its *CEN* localization. This implies that 2-μm plasmids have invented a new means to recruit Cse4 to their *STB* loci, which is probably key to their longevity. Hajra et al. (2006) speculate that a complex containing Rep1 and Rep2 may help deposit (and perhaps maintain) Cse4 at *STB*. This further implies that although some components of both the 2-μm plasmid and yeast kinetochores are bound to be in common, others (e.g., Ndc10) are not. Thus, the 2-μm plasmid kinetochore may prove to be valuable in future studies of *CEN*-based kinetochores.
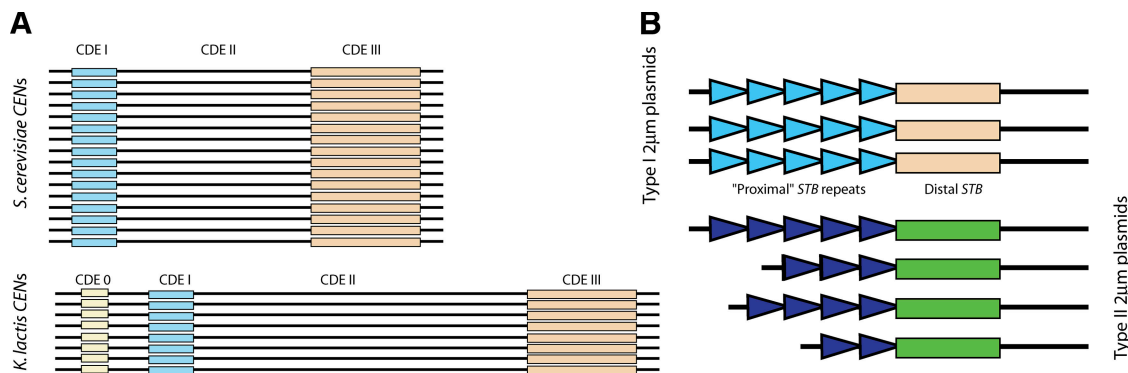

Figure 2. ***CEN* and *STB* evolve very differently.** (A) The 16 *S. cerevisiae* centromeres share many highly conserved features with each other and with the eight centromeres of *Kluyveromyces lactis*, indicating a high degree of sequence constraint, especially in CDE III. (B) Two types of 2-μm plasmids have been found in *S. cerevisiae*. Type I plasmids are quite similar to each other in size and sequence but are only 70% identical in sequence to type II plasmids, which also have a high degree of length variation that appears to bear functional consequences for 2-μm partitioning.

Centromere function is under extremely strong constraints in budding yeasts, and the general architecture of *CEN* sequences has been largely preserved over tens of millions of years (Fig. 2 A). In contrast, *STB* loci are highly variable in sequence and especially in length even within *S. cerevisiae* (Fig. 2 B; Rank et al., 1994b). Previous studies have strongly suggested that this is a result of antagonism between 2-μm plasmids and their host cells (Murray et al., 1988; Rank et al., 1994a), as the latter try to evolve genetic solutions to their 2-μm infestations. In addition, 2-μm plasmid alleles compete with each other for survival within yeast cells (Rank et al., 1994a). This means that Cse4 is forced to negotiate with both very slowly evolving (*CEN*) as well as extremely rapidly evolving (*STB*) centromeres, which is an interesting challenge for an essential histone.

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